In response to the May 2, 2005 Office Action, please amend the above application as follows:

Amendments To The Claims

The following listing of claims will replace all prior versions and listings of claims in this application. A clean set of the pending claims is attached hereto as **Exhibit A**.

Listing of Claims:

Claims 1-34 (canceled)

35. (currently amended) A method for producing a recombinant glycoprotein comprising an N-glycan structure that comprises a $Man_5GlcNAc_2$ glycoform in a lower eukaryotic host cell that does not display alpha-1,6 mannosyltransferase activity with respect to the N-glycan on a glycoprotein, the method comprising the step of introducing into the host cell a nucleic acid encoding an alpha-1,2 mannosidase enzyme selected to have optimal activity in the ER or Golgi of said host cell, the enzyme comprising:

(a) an alpha-1,2 mannosidase catalytic domain having [alpha-1,2 mannosidase activity selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in a subcellular location where the domain is targeted] optimal activity in said ER or Golgi at a pH between 5.1 and 8.0; fused to[; and]

(b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the mannosidase enzyme to the ER or Golgi apparatus of the host cell;

whereby, upon passage of the recombinant glycoprotein through the ER or Golgi apparatus

of the host cell, in excess of 30 mole % of the N-glycan structures attached thereto have a $Man_5GlcNAc_2$ glycoform that can serve as a substrate for GlcNAc transferase I *in vivo*.

36. – 38. (canceled)

39. (previously amended) The method of claim 35, wherein the mannosidase enzyme is targeted to the early, medial, late Golgi or the trans Golgi network of the host cell.

40. (previously amended) The method of claim 35, further comprising the step of introducing into the host cell one or more additional nucleic acids encoding one or more additional enzymes selected from the group consisting of mannosidases, glycosyltransferases and glycosidases.

41. – 43. (canceled)

44. (previously amended) The method of claim 35 or 40, wherein the recombinant glycoprotein comprising the N-glycan is further modified to comprise one or more sugars selected from the group consisting of N-acetylglucosamine, galactose, sialic acid and fucose.

45. (previously amended) The method of claim 35 or 40, wherein the recombinant glycoprotein comprising the N-glycan is further modified to comprise at least one oligosaccharide branch comprising the structure NeuNAc-Gal-GlcNAc-Man.

46. (previously amended) The method of any one of claims 35, 40, 70, 79 or 80, wherein the host is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia* sp.,

Saccharomyces cerevisiae, *Saccharomyces* sp., *Hansenula polymorpha*, *Kluyveromyces* sp., *Candida albicans*, *Aspergillus nidulans*, and *Trichoderma reesei*.

47. (previously amended) The method of claim 35 or 40, wherein the host further lacks the activity of one or more enzymes selected from the group consisting of mannosyltransferases and phosphomannosyltransferases.

48. (previously amended) The method of claim 47, wherein the host lacks an enzyme activity with respect to the N-glycan on a glycoprotein, the activity selected from the group consisting of 1,6 mannosyltransferase; 1,3 mannosyltransferase; and 1,2 mannosyltransferase.

49. (previously amended) The method of claim 35 or 40, wherein the host is an OCH1 mutant of *P. pastoris*.

50. (previously amended) The method of claim 35 or 40, wherein the host is genetically modified to express one or more enzymes selected from: GnTI; a UDP-specific diphosphatase; a GDP-specific diphosphatase; and a UDP-GlcNAc transporter.

51. (canceled)

52. (previously amended) The method of claim 35 or 40, further comprising the step of isolating the recombinant glycoprotein subsequent to passage of the recombinant glycoprotein through the ER or Golgi apparatus of the host cell.

53. (previously presented) The method of claim 52, further comprising the step of subjecting the isolated glycoprotein to at least one further glycosylation reaction *in vitro*, subsequent to its isolation from the host.

54. – 56. (canceled)

57. (currently canceled) [The method of claim 35, wherein the mannosidase enzyme has optimal activity at a pH between 5.1 and 8.0.]

58. (previously amended) The method of claim 35, wherein the mannosidase enzyme comprises an alpha-1,2-mannosidase catalytic domain from mouse, human, *Lepidoptera*, *Aspergillus nidulans*, *Xanthomonas manihotas* or *Bacillus* sp.

59. (previously amended) The method of claim 40, wherein at least one of the additional enzymes is localized in the host by forming a fusion protein between a catalytic domain of the enzyme and a cellular targeting signal peptide.

60. (previously presented) The method of claim 59, wherein the fusion protein is encoded by at least one genetic construct formed by the in-frame ligation of a DNA fragment encoding a cellular targeting signal peptide with a DNA fragment encoding a glycosylation enzyme or catalytically active fragment thereof.

61. (previously amended) The method of claim 59, wherein the catalytic domain encodes a glycosidase or glycosyltransferase selected from the group consisting of GnT I, GnT II, GnT III, GnT IV, GnT V, GnT VI, GalT, Fucosyltransferase and ST, and wherein the catalytic domain has optimal activity at a pH between 5.1 and 8.0.

62. (previously amended) The method of claim 35, further comprising the step of introducing into the host cell one or more additional nucleic acids encoding one or more additional enzymes selected from the group consisting of UDP-GlcNAc transferase, UDP-

galactosyltransferase, GDP-fucosyltransferase, CMP-sialyltransferase, UDP-GlcNAc transporter, UDP-galactose transporter, GDP-fucose transporter, CMP-sialic acid transporter, and nucleotide diphosphatases.

63. (previously amended) The method of claim 40, wherein the host is genetically modified to express GnTI and a UDP-GlcNac transporter.

64. (previously amended) The method of claim 40, wherein the host is genetically modified to express a UDP- or GDP-specific diphosphatase.

65. (previously presented) The method of claim 40, wherein the one or more additional enzymes is targeted to the endoplasmic reticulum, the early, medial or late Golgi, or the trans Golgi network of the host cell.

66. (previously presented) The method of claim 65, wherein the one or more additional enzymes is targeted by means of a cellular targeting signal peptide not normally associated with the enzyme.

67. (previously presented) The method of claim 40, wherein the one or more additional enzymes [is selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes where the enzyme is localized]has optimal activity at a pH between 5.1 and 8.0.

68. (previously amended) The method of claim 35 or 40, wherein a nucleic acid encoding one or more enzymes is introduced into the host cell by integration into the host cell chromosome.

69. (previously amended) The method of claim 40 or 68, wherein at least one of the encoded enzymes is GnTI.

70. (currently amended) A method for producing a recombinant glycoprotein comprising an N-glycan structure that comprises a $GlcNAcMan_5GlcNAc_2$ glycoform in a lower eukaryotic host cell, the cell genetically modified to produce N-glycan structures having an excess of 30 mole % of a $Man_5GlcNAc_2$ glycoform that can serve as a substrate for GlcNAc transferase I *in vivo*, the method comprising the step of introducing into said host cell a nucleic acid encoding a GlcNAc transferase I enzyme selected to have optimal activity in the ER or Golgi of said host cell, the enzyme comprising:

(a) a GlcNAc transferase I catalytic domain having optimal activity in said ER or Golgi at a pH between 5.1 and 8.0; fused to [GlcNAc transferase I activity selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in a subcellular location where the domain is targeted; and]

(b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the GlcNAc transferase I enzyme to the ER or Golgi apparatus of the host cell;

whereby, upon passage of the recombinant glycoprotein through the ER or Golgi apparatus of the host cell, a recombinant glycoprotein comprising a $GlcNAcMan_5GlcNAc_2$ glycoform is produced.

71. (currently amended) A method for producing a recombinant glycoprotein comprising an N-glycan structure that comprises a $GlcNAcMan_5GlcNAc_2$ glycoform in a lower eukaryotic host cell that does not display alpha-1,6 mannosyltransferase activity with respect to the

N-glycan on a glycoprotein, the method comprising the step or steps of introducing into the host cell one or more nucleic acids encoding at least two enzymes, the first enzyme selected to have optimal activity in the ER or Golgi of said host cell, the enzyme comprising:

(a) an alpha-1,2 mannosidase catalytic domain having optimal activity in said ER or Golgi at a pH between 5.1 and 8.0; fused to [alpha-1,2 mannosidase activity selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in a subcellular location where the domain is targeted; and]

(b) a cellular targeting signal peptide not normally associated with the catalytic domain of (a) and selected to target the first enzyme to the ER or Golgi apparatus of the host cell;

the second enzyme selected to have optimal activity in the ER or Golgi of said host cell, the enzyme comprising:

(c) a GlcNAc transferase I catalytic domain having optimal activity in said ER or Golgi at a pH between 5.1 and 8.0; fused to [GlcNAc transferase I activity selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in a subcellular location where the domain is targeted; and]

(d) a cellular targeting signal peptide not normally associated with the catalytic domain of (c) and selected to target the second enzyme to the ER or Golgi apparatus of the host cell;

whereby, upon passage of the recombinant glycoprotein through the ER or Golgi apparatus of the host cell, a recombinant glycoprotein comprising a $GlcNAcMan_5GlcNAc_2$ glycoform is produced.

72. (previously amended) The method of any one of claims 70, 71, 79 and 80, further comprising the step of introducing into the host cell a nucleic acid encoding a UDP-GlcNAc transporter.

73. (previously amended) The method of any one of claims 35, 40, 70, 71, 79 or 80, further comprising the step of analyzing a glycosylated protein or isolated *N*-glycan produced in the host cell by one or more methods selected from the group consisting of: (a) mass spectroscopy; (b) liquid chromatography; (c) characterizing cells using a fluorescence activated cell sorter, spectrophotometer, fluorimeter, or scintillation counter; (d) exposing host cells to a lectin or antibody having a specific affinity for a desired oligosaccharide moiety; and (e) exposing cells to a cytotoxic or radioactive molecule selected from the group consisting of sugars, antibodies and lectins.

74. – 78. (not entered)

79. (currently amended) A method for producing a recombinant glycoprotein comprising an N-glycan comprising a $GlcNAcMan_3GlcNAc_2$ glycoform in a lower eukaryotic host cell genetically modified to produce N-glycan structures having an excess of 30 mole % of a $Man_5GlcNAc_2$ glycoform that are converted *in vivo* to a $GlcNAcMan_5GlcNAc_2$ glycoform by GlcNAc transferase I activity localized in the ER or Golgi apparatus of the host cell, the method comprising the step of introducing into the host cell a nucleic acid encoding a mannosidase II enzyme <u>selected to have optimal activity in the ER or Golgi of said host cell, the enzyme</u> comprising:

(a) a mannosidase II catalytic domain having optimal activity in said ER or Golgi at a pH between 5.1 and 8.0; fused to [mannosidase II activity selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in a subcellular location where the domain is targeted; and]

(b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the mannosidase II enzyme to the ER or Golgi apparatus of the host cell;

whereby, upon passage of the recombinant glycoprotein through the ER or Golgi apparatus of the host cell, a recombinant glycoprotein comprising a $GlcNAcMan_3GlcNAc_2$ glycoform is produced.

80. (currently amended) A method for producing a recombinant glycoprotein comprising an N-glycan comprising a $GlcNAc_2Man_3GlcNAc_2$ glycoform in a lower eukaryotic host cell genetically modified to produce N-glycan structures having an excess of 30 mole % of a $Man_5GlcNAc_2$ glycoform that are converted *in vivo* to a $GlcNAcMan_3GlcNAc_2$ glycoform by GlcNAc transferase I and mannosidase II localized in the ER or Golgi apparatus of the host cell, the method comprising the step of introducing into the host cell a nucleic acid encoding a GlcNAc transferase II enzyme selected to have optimal activity in the ER or Golgi of said host cell, the enzyme comprising:

(a) a GlcNAc transferase II catalytic domain having optimal activity in said ER or Golgi at a pH between 5.1 and 8.0; fused to [GlcNAc transferase II activity selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in a subcellular location where the domain is targeted; and]